

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (Canceled)

2 - 54. (Canceled)

55. (New) A serum-free, eukaryotic cell culture medium comprising the ingredients N-acetyl-L-cysteine, 2-mercaptoethanol, human serum albumin, D,L-tocopherol acetate, soluble human lipids for serum-free media, ethanolamine, human zinc insulin, iron-saturated transferrin, Se^{4+} , hydrocortisone, Ca^{2+} , K^{+} , Mg^{2+} , Na^{+} , CO_3^{2-} , PO_4^{3-} , D-glucose, HEPES, sodium pyruvate, phenol red, glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-arginine HCl, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, D-calcium pantothenate, choline chloride, folic acid, i-inositol, niacinamide, pyridoxal HCl, riboflavin, thiamine HCl, and vitamin B₁₂,

wherein each of said ingredients is present in said medium at a concentration that supports the expansion of CD34⁺ hematopoietic cells in suspension culture in the absence of stromal cells.

56. (New) The serum-free, eukaryotic cell culture medium according to claim 55, wherein said medium is obtained by combining water and the ingredients of claim 55.

57. (New) A method of making a serum-free, eukaryotic cell culture medium, said method comprising admixing water and the ingredients according to claim 55.

58. (New) The medium obtained by the method of claim 57.

59. (New) A method of expanding CD34⁺ hematopoietic cells, said method comprising:

- (a) contacting said cells with the serum-free medium of claim 58; and
- (b) culturing said cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of said cells.

60. (New) The method of claim 59, wherein said cells are recombinant CD34⁺ hematopoietic cells.

61. (New) A method of providing recombinant CD34⁺ hematopoietic cells to a mammal, said method comprising:

- (a) expanding recombinant CD34⁺ hematopoietic cells according to the method of claim 60; and
- (b) introducing said recombinant cells into said mammal.

62. (New) A method of expanding CD34⁺ hematopoietic cells, said method comprising:

- (a) contacting said cells with the serum-free medium of claim 55; and
- (b) culturing said cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of said cells.

63. (New) The method of claim 62, wherein said cells are recombinant CD34⁺ hematopoietic cells.

64. (New) A method of providing recombinant CD34⁺ hematopoietic cells to a mammal, said method comprising:

- (a) expanding recombinant CD34⁺ hematopoietic cells according to the method of claim 63; and
- (b) introducing said recombinant cells into said mammal.

65. (New) A serum-free, eukaryotic cell culture medium, wherein said medium is a 1X medium, wherein said medium contains the following ingredients at the following concentrations:

Ingredient	Concentration Range (mg/L) (About)
CaCl ₂	1 - 500
KCl	1 - 500
KNO ₃	0.008 - 0.8

MgSO ₄	10 - 500
NaCl	3000 - 9000
NaHCO ₃	100 - 4000
NaH ₂ PO ₄ • water	10 - 750
L-Alanine	5 - 250
L-Asparagine (free base)	5 - 150
L-Arginine HCl	10 - 250
L-Aspartic Acid	5 - 125
L-Cystine 2 • HCl	1 - 200
L-Glutamic Acid	5 - 500
Glycine	5 - 200
L-Histidine • HCl • water	5 - 200
L-Isoleucine	5 - 500
L-Leucine	25 - 500
L-Methionine	5 - 500
L-Phenylalanine	5 - 500
L-Proline	5 - 500
L-Serine	5 - 500
L-Threonine	5 - 500
L-Lysine • HCl	25 - 500
L-Tryptophan	2 - 100
L-Tyrosine (disodium salt)	25 - 500
L-Valine	5 - 500
Biotin	0.01 - 1.0
D-Ca Pantothenate	0.05 - 10.0
Choline Chloride	1 - 150
Folic Acid	0.1 - 10.0
i-Inositol	1 - 75
Niacinamide	0.1 - 10.0

Pyridoxal • HCl	0.1 - 10.0
Riboflavin	0.01 - 2.0
Thiamine • HCl	0.1 - 10.0
Vitamin B ₁₂	0.001 - 5.0
D-Glucose	2000 - 9000
Phenol Red	0.5 - 30
HEPES	1000 - 7000
Sodium Pyruvate	10 - 300
Soluble human lipids for serum-free media	1 - 15
Ethanolamine	1 - 25
Iron Saturated Human Transferrin	10 - 500
N-acetyl-L-cysteine	16 - 660
2-Mercaptoethanol	2 - 8,

and wherein said medium supports the expansion of CD34⁺ cells in serum-free culture.

66. (New) The serum free, eukaryotic cell culture medium of claim 65, wherein said medium supports the expansion of CD34⁺ cells in suspension culture.

67. (New) The serum-free, eukaryotic cell culture medium of claim 65, wherein said medium supports the expansion of CD34⁺ hematopoietic cells in the absence of stromal cells.

68. (New) The serum-free, eukaryotic cell culture medium according to claim 65, wherein said ingredients in said 1X medium are present at the following concentrations:

Ingredient	Concentration (mg/L) (About)
CaCl ₂	165
KCl	330
KNO ₃	0.08
MgSO ₄	100
NaCl	4,500
NaHCO ₃	3,000
NaH ₂ PO ₄ • water	125
L-Alanine	25
L-Asparagine (free base)	25
L-Arginine HCl	84
L-Aspartic Acid	30
L-Cystine 2 • HCl	90
L-Glutamic Acid	75
Glycine	30
L-Histidine • HCl • water	42
L-Isoleucine	105
L-Leucine	105
L-Methionine	30
L-Phenylalanine	70
L-Proline	40
L-Serine	40
L-Threonine	100
L-Lysine • HCl	150
L-Tryptophan	15
L-Tyrosine (disodium salt)	100
L-Valine	95
Biotin	0.01
D-Ca Pantothenate	4

Choline Chloride	4
Folic Acid	4.00
i-Inositol	7
Niacinamide	4
Pyridoxal • HCl	4
Riboflavin	0.4
Thiamine • HCl	4
Vitamin B ₁₂	0.001
D-Glucose	4500
Phenol Red	15
HEPES	6000
Sodium Pyruvate	110
Soluble human lipids for serum-free media	5
Ethanolamine	10
Iron Saturated Human Transferrin	100
N-acetyl-L-cysteine	160
2-Mercaptoethanol	4.

69. (New) A method of expanding CD34⁺ hematopoietic cells, said method comprising:

- (a) contacting said cells with the serum-free medium of claim 68; and
- (b) culturing said cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of said cells.

70. (New) A method of providing recombinant CD34⁺ hematopoietic cells to a mammal, said method comprising:

- (a) expanding said recombinant CD34⁺ hematopoietic cells according to the method of claim 69; and
- (b) introducing said recombinant cells into said mammal.

71. (New) A method of expanding recombinant CD34⁺ hematopoietic cells in serum-free culture, said method comprising expanding said recombinant cells according to the method of claim 69.

72. (New) A method of expanding CD34⁺ hematopoietic cells, said method comprising:

- (a) contacting said cells with the serum-free medium of claim 65; and
- (b) culturing said cells in serum-free suspension culture, in the absence of stromal cells, under conditions that facilitate the expansion of said cells.

73. (New) A method of expanding recombinant CD34⁺ hematopoietic cells in serum-free culture, said method comprising expanding said recombinant cells according to the method of claim 72.

74. (New) A method of providing recombinant CD34⁺ hematopoietic cells to a mammal, said method comprising:

- (a) expanding said recombinant CD34⁺ hematopoietic cells according to the method of claim 72; and
- (b) introducing said recombinant cells into said mammal.

75. (New) A serum-free, eukaryotic cell culture medium, wherein said medium is a 1X medium prepared by admixing water and the following ingredients at the following concentrations:

Ingredient	Concentration Range (mg/L) (About)
CaCl ₂	1 - 500
KCl	1 - 500
KNO ₃	0.008 - 0.8
MgSO ₄	10 - 500
NaCl	3000 - 9000
NaHCO ₃	100 - 4000
NaH ₂ PO ₄ • water	10 - 750
L-Alanine	5 - 250
L-Asparagine (free base)	5 - 150
L-Arginine HCl	10 - 250
L-Aspartic Acid	5 - 125
L-Cystine 2 • HCl	1 - 200
L-Glutamic Acid	5 - 500
Glycine	5 - 200
L-Histidine • HCl • water	5 - 200
L-Isoleucine	5 - 500
L-Leucine	25 - 500
L-Methionine	5 - 500
L-Phenylalanine	5 - 500
L-Proline	5 - 500
L-Serine	5 - 500
L-Threonine	5 - 500
L-Lysine • HCl	25 - 500

L-Tryptophan	2 - 100
L-Tyrosine (disodium salt)	25 - 500
L-Valine	5 - 500
Biotin	0.01 - 1.0
D-Ca Pantothenate	0.05 - 10.0
Choline Chloride	1 - 150
Folic Acid	0.1 - 10.0
i-Inositol	1 - 75
Niacinamide	0.1 - 10.0
Pyridoxal • HCl	0.1 - 10.0
Riboflavin	0.01 - 2.0
Thiamine • HCl	0.1 - 10.0
Vitamin B ₁₂	0.001 - 5.0
D-Glucose	2000 - 9000
Phenol Red	0.5 - 30
HEPES	1000 - 7000
Sodium Pyruvate	10 - 300
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Ethanolamine	1 - 25
Iron Saturated Human Transferrin	10 - 500
N-acetyl-L-cysteine	16 - 660
2-Mercaptoethanol	2 - 8,

and wherein said medium supports the expansion of CD34⁺ cells in serum-free culture.